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Research Note

Prevalence of Antibiotic-Resistant Fecal *Escherichia coli* Isolates from Penned Broiler and Scavenging Local Chickens in Arusha, Tanzania

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ABSTRACT

We compared the prevalence of antibiotic-resistant *Escherichia coli* isolates from household-level producers of broiler (commercial source breeds) and local chickens in the Arusha District of Tanzania. Households were composed of a single dwelling or residence with independent, penned broiler flocks. Free-range, scavenging chickens were mixed breed and loosely associated with individual households. A total of 1,800 *E. coli* isolates (1,200 from broiler and 600 from scavenging local chickens) from 75 chickens were tested for their susceptibility against 11 antibiotics by using breakpoint assays. Isolates from broiler chickens harbored a higher prevalence of antibiotic-resistant *E. coli* relative to scavenging local chickens, including sulfamethoxazole (80.3 versus 34%), followed by trimethoprim (69.3 versus 27.7%), tetracycline (56.8 versus 20%), streptomycin (52.7 versus 24.7%), amoxicillin (49.6 versus 17%), ampicillin (49.1 versus 16.8%), ciprofloxacin (21.9 versus 1.7%), and chloramphenicol (1.5 versus 1.2%). Except for resistance to chloramphenicol, scavenging local chickens harbored fewer resistant *E. coli* isolates ($P < 0.05$). Broiler chickens harbored more isolates that were resistant to ≥ 7 antibiotics ($P < 0.05$). The higher prevalence of antibiotic-resistant *E. coli* from broiler chickens correlated with the reported therapeutic and prophylactic use of antibiotics in this poultry population. We suggest that improved biosecurity measures and increased vaccination efforts would reduce reliance on antibiotics by these households.

Key words: Antibiotic resistance; Arusha; Chickens; *Escherichia coli*; Tanzania

The emergence of antibiotic resistance in bacteria is a global challenge, and use of antibiotics in food-animal agriculture is considered a significant contributor to this problem *(17, 25, 33)*. In Tanzania, most households that keep livestock also keep chickens for meat production (either commercial broiler breeds or scavenging local breeds). Even though the commercial breeds are being raised at the household level (private owners, single dwelling, or residence), they are typically exposed to antibiotics for prophylactic, therapeutic, and growth promotion purposes *(22)*.

Handling and consumption of raw or undercooked contaminated poultry meat is a potential risk factor for the transmission of bacteria, including antibiotic-resistant strains *(14)*. *Escherichia coli* is a commensal enteric bacterium that is often used as an indicator organism to study the prevalence and distribution of antibiotic resistance for other enteric bacteria in animals and people *(2)*. Although not pathogenic, commensal *E. coli* often harbors resistance traits that can be transferred to infectious pathogens *(3, 11, 13, 17, 21, 27)*. Fecal contamination of carcasses during slaughtering can increase the probability of spreading antibiotic-resistant bacteria to people *(2, 5)*.

Note that ingestion of antibiotic-resistant bacteria by people and animals can result in colonization of resistant bacteria even in the absence of antibiotic selection pressure *(19)*. Some antibiotics that are used in veterinary medicine are also clinically important in human medicine *(6)*. Consequently, infections caused by these foodborne-resistant isolates or infections caused by other pathogens that acquire resistance traits from animal-borne isolates likely contribute to this health and economic challenge *(7)*.

To address these challenges, particularly in developing countries, it is important to identify the factors that drive the emergence and dissemination of antibiotic-resistant bacteria in people and animals. Poultry are a growing source of animal protein in low-income countries, such as Tanzania, but increasing demand can only be met by using more input intensive husbandry systems. The goal of this project was to contrast a more traditional free-range system with a growing commercial breed husbandry strategy in a peri-urban area of Arusha, Tanzania. Scavenging local chickens are essentially mixed-breed, indigenous chickens that scavenge food resources and receive no commercial food inputs. Thus,

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these chickens require relatively low resource input and opportunistic yield, whereas commercial breed chickens are purchased by households and are typically raised within enclosures and provided commercial feed. Antibiotics are often mixed into the feed and water of these birds. Comparing these two systems gives us information about how changing from more traditional practices to more input-intensive practices might impact the prevalence of antibiotic-resistant bacteria in peri-urban flocks. An increasing prevalence of resistant bacteria with commercial breeds could represent an important public health challenge, as more households invest in these systems.

MATERIALS AND METHODS

Sampling strategy. A cross-sectional study was conducted from June to September 2015 to estimate the prevalence of antibiotic-resistant *E. coli* from meat chickens in Arusha, Tanzania. Five wards (Mifugo, Nambala, Njiro, Sakina, and Sansi) were identified. From each ward, we selected one scavenging local and one penned broiler chicken–producing household by convenience sampling. Fresh fecal droppings were collected from both scavenging local ($\leq$20 per household) and penned broiler ($\leq$200 per household) chickens from each ward. A total of 75 fresh fecal droppings ($n = 5$ scavenging local and $n = 10$ penned broiler chicken fecal droppings per household) were collected in sterile plastic bags and were transported to the laboratory ($\leq$2 h) on ice.

Fecal sample processing for isolation of *E. coli*. Upon reception by the laboratory at the Nelson Mandela African Institution of Science and Technology (NM-AIST), the fecal samples were suspended in sterile distilled water (1:9 ratio, feces:water). Fecal suspensions (100 μl) were serially diluted (10-fold) with sterile distilled water, and 30 μl of each dilution were plated onto 25-mm-diameter MacConkey agar plates (BD, Sparks, MD) by using sterile glass beads and incubated overnight at 37°C for isolation of putative *E. coli* isolates. After incubation, up to 24 isolates were transferred into 150 μl of Luria-Bertani broth (BD) in 96-well plates and incubated again overnight at 37°C. After an overnight incubation, sterile glycerol (15% final concentration) was added to each well and stored at −80°C for further analyses.

Determining antibiotic susceptibility. Each *E. coli* isolate was tested against 11 antibiotics by using a breakpoint assay *(30)*. Briefly, MacConkey agar was mixed with each antibiotic at a fixed concentration that was guided by the Clinical and Laboratory Standards Institute *(4)*. The *E. coli* cultures from 96-well plates were transferred (approximately 2 μl each with a sterile 96-pin replicator) onto 50-mm-diameter MacConkey agar plates containing antibiotics and were then incubated overnight at 37°C along with antibiotic-resistant control strains (*E. coli* NM-1 and *E. coli* NM-2, both originally isolated at NM-AIST) and an antibiotic-susceptible *E. coli* K-12 strain (ATCC.org). The NM-1 strain was resistant to ampicillin (Amp), ciprofloxacin (Cip), chloramphenicol (Chm), streptomycin (Str), sulfamethoxazole (Sul), tetracycline (Tet), and trimethoprim (Tri), and the NM-2 strain was resistant to amoxicillin (Amx), ceftazidime (Cfd), cefotaxime (Ctx), and gentamicin (Gen). After incubation, the plates were examined for the presence (resistant) or absence (susceptible) of the growth of *E. coli* isolates. The numbers of resistant isolates were enumerated, and the prevalence of antibiotic-resistant phenotypes was calculated.

The 11 antibiotics included β-lactams, cephalosporins, amphenicol, Tet, sulfonamides, aminoglycosides, and fluoroquinolones. These included Amp (32 μg/ml; VWR International LLC, Sanborn, NY), Amx (32 μg/ml; MP Biomedicals LLC, Solon, OH), Ctx (8 μg/ml; Chem-Impex, International Inc., Wood Dale, IL), Cfd (8 μg/ml; Sigma-Aldrich Co., St. Louis, MO), Chm (32 μg/ml; Mediatech Inc., Manassas, VA), Cip (4 μg/ml; Enzo Life Sciences Inc., Farmingdale, NY), Gen (64 μg/ml; Mediatech Inc.), Tet (16 μg/ml; MP Biomedicals LLC), Tri (8 μg/ml; MP Biomedicals LLC), Sul (512 μg/ml; MP Biomedicals LLC), and Str (16 μg/ml; Amresco Inc., Solon, OH).

Statistical analysis. Antibiotic resistance data were treated as a binary variable (1 = resistant; 0 = susceptible). Descriptive statistics were performed by using Access (Microsoft Office 2007, Microsoft Corporation, Redmond, WA). Average prevalence for different groups was compared using one-way analysis of variance with a Tukey-Kramer pairwise postcomparison test. A *t* test was used for pairwise comparison of overall prevalence of resistant *E. coli* isolates between scavenging local and broiler-type chickens.

RESULTS

Broiler chickens harbor a higher prevalence of antibiotic-resistant *E. coli*. A total of 1,800 *E. coli* isolates were collected from the fecal samples ($n = 600$ scavenging local chickens and $n = 1{,}200$ broiler chickens). Penned broiler chickens harbored more *E. coli* that was resistant to $\geq$1 antibiotic compared with scavenging local chickens ($P < 0.001$). In both scavenging local and penned broiler chickens, *E. coli* isolates harbored relatively higher resistance to Sul. The rank order of resistance in scavenging local chickens was Sul, Tri, Str, Tet, Amx, Amp, Cip, and Chm. In broiler chickens, a very similar order was evident except that Tet and Str were reversed (Table 1). For individual antibiotics, broiler chickens harbored a significantly ($P < 0.05$) higher proportion of resistance for the previously listed antibiotics except for Chm. No resistance was detected for Cfd, Ctx, and Gen. In addition, broiler chicken isolates collected from the Sakina ward harbored a greater proportion of Tri resistance compared with the Njiro ward ($P < 0.05$). In scavenging local chicken's isolates, the proportion of resistance between wards was significantly different for Amp and Amx, with Amp and Amx being significantly higher in the Nambala ward compared with the Mifugo, Njiro, and Sansi wards ($P < 0.05$; Table 1).

Broiler chickens harbored more unique multidrug resistance phenotypes. A total of 52 and 105 unique multidrug resistance phenotypes ($\geq$2 antibiotics) were detected from scavenging local and broiler chickens, respectively (Table 2). The percentages of *E. coli* isolates resistant for two to three and four to six drugs were relatively similar between scavenging local and broiler chickens' isolates (Table 3), but broiler chickens harbored a higher proportion of isolates that were resistant to seven to eight antibiotics (Table 3). The most frequent multidrug resistance phenotype found in scavenging local chickens was SulTri, and in broiler chickens was AmpAmxStrSulTetTri (Table 2). The broadest resistance phenotypes were AmpAmxChmStrSulTetTri and AmpAmxCipStrSulTetTri in scavenging local chickens and AmpAmxChmCipStrSulTetTri in broiler chickens (Table 2).

TABLE 1. *Prevalence of antibiotic-resistant fecal* E. coli *from scavenging local and penned broiler chickens collected from different wards in Arusha, Tanzania*

Ward	Amp[a]	Amx	Chm	Cip	Str	Sul	Tet	Tri	SE[b]
Scavenging local									
Nambala	39.2	39.2	4.2	0.0	30.8	48.3	23.3	44.2	3.1
Njiro	12.5	13.3	0.0	4.2	23.3	24.2	44.2	22.5	2.5
Mifugo	10.0	10.0	1.7	2.5	18.3	20.8	16.7	16.7	3.0
Sakina	16.7	16.7	0.0	0.0	35.8	29.2	3.3	24.2	4.9
Sansi	5.8	5.8	0.0	1.7	15.0	47.5	12.5	30.8	4.6
SE	5.9	5.8	0.8	0.8	3.9	5.8	6.9	4.7	
Broilers									
Nambala	36.7	36.7	0.8	30.0	58.8	77.1	67.5	78.8	0.4
Njiro	48.8	50.8	5.0	25.8	67.1	70.4	53.8	47.9	0.5
Mifugo	43.8	44.6	0.4	7.5	29.2	82.9	39.2	65.0	0.8
Sakina	67.9	65.4	0.8	27.1	56.3	80.4	64.2	84.2	0.3
Sansi	48.3	50.4	0.4	19.2	52.1	90.8	59.2	70.4	0.3
SE	5.2	4.7	0.9	4.0	6.4	3.4	5	6.3	
P value[c]	0.003	0.002	0.79	0.001	0.005	0.0001	0.002	<0.001	

[a] Amp, ampicillin; Amx, amoxicillin; Chm, chloramphenicol; Cip, ciprofloxacin; Str, streptomycin; Sul, sulfamethoxazole; Tet, tetracycline; Tri, trimethoprim. No resistance was detected for ceftazidime (Cfd), cefotaxime (Ctx), or gentamicin (Gen).
[b] SE, standard error for average of resistance between samples in different wards.
[c] *P* value, comparison of prevalence of individual antibiotic resistance in scavenging local versus broiler chickens.

DISCUSSION

For this study, we assumed that free-range scavenging chickens are, on average, rarely exposed to antibiotics, whereas broilers are routinely exposed to antibiotics. Qualitatively, our findings are consistent with *E. coli* from scavenging local chickens experiencing less frequent antibiotic selection pressure compared with the broiler chickens from Arusha. We detected a higher prevalence of resistance to Sul, Str, Tri, and Tet among chicken isolates, especially in broilers, which is consistent with earlier reports indicating that Tet, sulfonamides, and Tri are frequently used in broiler production in Africa *(23, 24)*. These drugs are easily available without prescription and are considered more efficient and less expensive in Africa *(28, 29)*. In most cases, sulfonamide drugs are used in combination with Tri (Sul-Tri). Similar findings of higher prevalence of Sul, Str, and Tet resistance among poultry *E. coli* were also reported in other studies conducted in Poland, British Columbia, and Tunisia *(8, 20, 29)*. The low prevalence of Chm resistance found in this study in both scavenging local and broiler chickens and other studies is consistent with little or no use of this antibiotic in our study area, and we did not observe any commercial Chm or florfenicol products in use by the farmers *(10, 12, 26)*.

The prevalence of Cip-resistant *E. coli* was higher in broiler compared with scavenging local chickens but still lower compared with other studies *(2, 18)*. Although these drugs are used frequently in well-organized large commercial farms, the relatively high cost of Cip probably limits the use in household-raised broiler chickens. Resistance to fluoroquinolones is often mediated by chromosomal point mutations and can easily persist in the environment for extended periods of time *(34)*. Consequently, persistence increases the likelihood that these resistant strains can be successfully transmitted to the people and other animals associated with these chickens. For instance, Johnson et al. *(13)* found that human *E. coli* isolates resistant to Cip were very similar to chicken isolates, a conclusion that was bolstered by molecular evidence that chickens were a source of pathogenic fluoroquinolone–resistant *E. coli* colonizing people. Another study found a correlation between the prevalence of Amx-resistant bacteria in broilers and broiler farmers *(32)*. Therefore, the presence of resistant traits in these populations suggests a risk to the households and consumers.

We found no strains with resistance to Gen, Ctx, and Cfd. Similar findings were reported in other studies *(15, 26)*. Gen and cephalosporins are frequently used to treat human infections; therefore, the lack of detectable prevalence of resistance for these drugs in household poultry *E. coli* isolates is a positive outcome from this investigation *(16)*.

In both broilers and free-range chickens, Sul resistance appeared to be associated with Str resistance in quite a number of resistance phenotypes. In a previous study, genes that conferred resistance to Str (*strA-strB*) were linked to a gene that conferred resistance to sulfonamides (*sul2*) and were often carried in the RSF1010 plasmid *(28)*. In addition, these resistant determinants can also be carried on transposons *(17)*.

If our assumptions about antibiotic exposure are correct, the presence of resistant *E. coli* in scavenging local chickens illustrates that antibiotic use is not requisite to the persistence of resistance traits *(1)*. Other factors, such as free grazing from a contaminated environment and from the spillage of broiler-raising households, might impact the level of antibiotic resistance in scavenging local chickens. The environment can also be a potential reservoir of resistant bacteria and resistant genes *(33)*. Therefore, it is possible that scavenging local chickens gained unintended access to

TABLE 2. *Prevalence of* E. coli *isolates from broilers (*n = *1,200) and scavenging local chickens (*n = *600) expressing different multidrug resistance (MDR) phenotypes*

MDR phenotype[a]	Broiler chickens	Scavenging local chickens
Susceptible	4.00	48.33
Tri	0.92	1.33
Str	0.75	3.67
Cip	0.17	0.17
Sul	1.42	3.67
Tet	1.17	6.67
Amp	0.67	0.33
Amx	0.75	0.50
Chm	0.08	—[b]
StrTri	0.58	1.00
CipTri	0.33	—
StrTet	1.08	—
StrSul	2.58	1.00
SulTri	4.17	5.17
TetTri	0.67	0.50
SulTet	1.17	1.00
CipTet	—	0.17
AmxStr	0.33	—
AmpTri	0.17	—
AmxTri	0.08	0.67
AmpStr	0.25	—
AmpTet	0.25	—
AmxTet	0.33	0.17
AmpSul	1.17	0.33
AmxCip	0.08	—
AmxSul	0.33	0.50
AmpAmx	0.75	1.00
StrSulTri	4.42	2.33
StrTetTri	1.17	0.17
CipSulTri	0.33	—
StrSulTet	1.08	0.17
SulTetTri	4.75	3.17
CipStrTet	0.17	—
CipSulTet	0.42	—
AmxStrTri	0.08	—
AmxSulTri	1.58	0.17
AmxTetTri	0.08	0.17
AmpStrTri	0.33	—
AmpSulTri	0.83	0.67
AmpTetTri	0.25	—
AmxCipTri	0.08	—
ChmCipTri	0.08	—
AmxStrSul	0.33	0.33
AmpStrTet	0.08	0.17
AmpStrSul	0.08	—
AmpCipTet	0.08	—
AmpSulTet	1.33	—
AmxSulTet	0.17	0.17
AmpCipSul	0.33	—
StrSulTetTri	3.17	0.67
CipStrSulTri	0.33	—
AmpAmxTri	0.67	0.33
AmpAmxStr	0.25	0.17
AmpAmxTet	0.67	0.17
AmpAmxSul	1.75	0.50
CipSulTetTri	1.08	—
CipStrSulTet	0.17	—
AmxSulTetTri	2.42	—
AmxCipTetTri	0.17	—
AmxStrSulTet	0.25	—
AmxCipSulTri	0.08	—
AmxCipStrSul	0.08	—
ChmStrSulTet	—	0.17
ChmSulTetTri	0.08	—
AmpSulTetTri	2.08	0.67
AmpStrSulTet	0.67	—
AmpCipTetTri	0.08	—
AmpCipStrSul	0.08	—
AmxCipSulTet	0.25	—
AmpCipSulTet	0.58	—
AmpAmxTetTri	0.33	—
AmpAmxStrTri	0.25	—
AmxChmSulTri	0.08	—
CipStrSulTetTri	3.08	0.33
AmpAmxSulTri	4.17	3.17
AmpAmxStrSul	1.67	2.17
AmpAmxStrTet	0.17	—
AmpAmxSulTet	0.50	0.33
AmpAmxCipTet	0.08	0.17
AmpStrSulTetTri	0.42	0.33
AmxStrSulTetTri	1.00	0.17
ChmCipStrSulTri	0.08	—
AmxCipStrSulTri	0.08	0.17
AmxCipStrTetTri	0.08	—
AmpAmxChmCip	0.08	—
AmpCipStrSulTri	0.17	—
AmpCipStrSulTet	0.17	—
AmxCipSulTetTri	1.17	—
AmxCipStrSulTet	0.17	—
AmpAmxStrSulTri	3.58	0.67
AmpAmxStrTetTri	0.25	—
AmpChmStrSulTri	—	0.17
AmxChmStrSulTri	0.08	0.17
AmpAmxSulTetTri	4.50	2.00
AmpAmxCipTetTri	0.08	0.17
AmpAmxStrSulTet	1.00	0.17
AmpAmxCipSulTet	0.25	—
AmpAmxChmStrSul	—	0.17
AmpCipStrSulTetTri	0.08	—
AmxCipStrSulTetTri	0.50	—
AmpAmxChmCipStr	0.08	—
AmpAmxChmSulTet	0.08	—
AmpChmStrSulTetTri	—	0.17
AmpChmCipStrSulTri	0.08	—
AmpAmxCipStrSulTri	0.25	0.17
AmpAmxCipStrTetTri	0.17	—
AmpAmxStrSulTetTri	6.58	1.50
AmpAmxCipSulTetTri	2.67	0.17
AmpAmxCipStrSulTet	0.33	—
AmpAmxChmStrSulTri	—	0.17
AmpAmxCipStrSulTetTri	6.42	0.17
AmxChmCipStrSulTetTri	0.08	—
AmpAmxChmStrSulTetTri	0.08	0.17
AmpAmxChmCipStrSulTri	0.08	—
AmpAmxChmCipStrSulTetTri	0.42	—

[a] For abbreviations, see Table 1, footnote *a*, and the text.

[b] —, no resistance was observed for the corresponding MDR phenotype.

TABLE 3. *Prevalence of multidrug resistance phenotypes among fecal* E. coli *isolates from scavenging local and broiler chickens in Arusha, Tanzania*

No. of antibiotics to which isolates were resistance	Scavenging local chickens, % ($n = 212$)	Broilers chickens, % ($n = 1,088$)	*P* value
2–3	56.5	38.5	0.064
4–6	41.9	53.2	0.596
7–8	0.8	8.2	0.019

the medicated feeds or antibiotic-contaminated feed and water.

When interviewed, the broiler farmers from our study admitted using antibiotics to prevent and treat diseases, such as colibacillosis, diarrhea, fowl typhoid, Newcastle disease, and unfamiliar disease symptoms. Farmers reported giving antibiotics to entire flocks after observing one or a few sick chickens. Although few farmers admitted to following the manufacturer's instructions when using antibiotics, most of them used advice from veterinary drug sellers and made decisions based on past experience. During interviews, we observed improper dosing (over and under) and the use of expired antibiotics. It is likely these practices collectively facilitate a higher prevalence of antibiotic-resistant *E. coli* in broiler chickens compared with scavenging local chickens. Given the cost of these inputs, it is highly unlikely that the farmers in this region would use antibiotics if disease issues were not evident. Consequently, reducing the incidence of disease should be a leading strategy to reduce reliance on antibiotics. For instance, biosecurity measures, such as vaccination, hygiene improvement, use of clean water, and limiting overcrowding of chickens, can minimize the probability of disease outbreaks *(9, 31)*. We propose that these investments will lessen the necessity of chicken farmers to rely on antibiotics for economic success.

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